BAC sequencing analyses in the hexaploid *Spartina maritima* (Poaceae): Homoeolog divergence and microsynteny in the grass family

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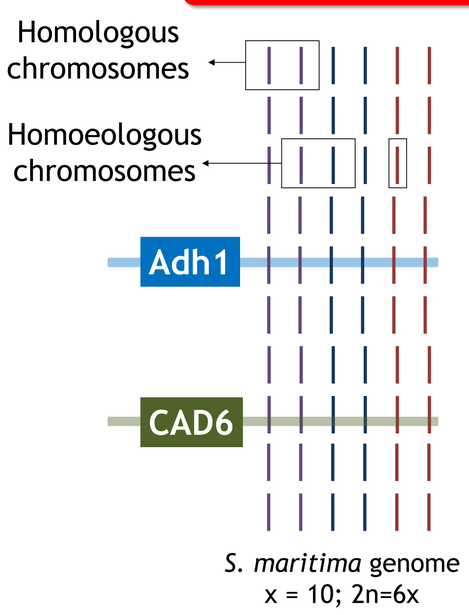
INTRODUCTION

Spartina maritima

Spartina maritima is a hexaploid species (2n=6x=60, 2C=3700 Mb) native to the European and African Atlantic coasts, which hybridized with different *Spartina* species introduced from America, contributing to the formation of new hybrids and invasive allopolyploid species. Although the autopolyploid or allopolyploid origin of the hexaploid *Spartina* lineage is not fully ascertained, nuclear gene phylogeny (Fortune et al. 2007) and the prominence of interspecific hybridization in this genus (Ainouche et al. 2012) suggest a hybrid origin. Thus, up to three duplicated (homoeologous) genomes may be expected in *S. maritima*.

In order to analyze homoeologous regions and to evaluate their molecular divergence, we used a Bacterial Artificial Chromosome (BAC) library constructed in *S. maritima*. Ten BAC clones containing two target chromosomal regions were analyzed and compared among representatives from other grass subfamilies.

MATERIAL & METHODS

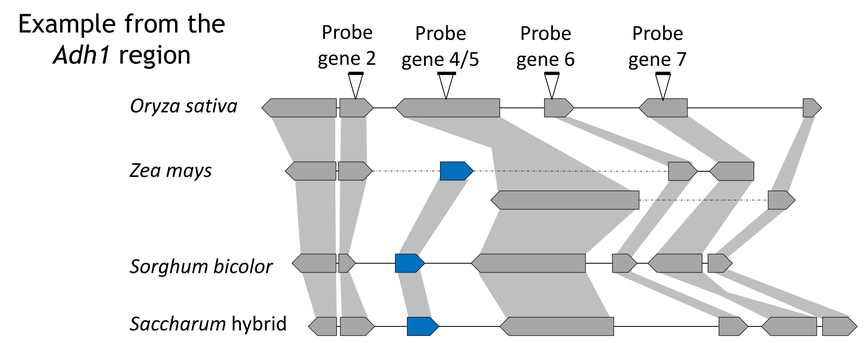


Construction of the *S. maritima* BAC library (CNRGV, Toulouse):
Genomic DNA extracted from plants sampled in southern Brittany (Morbihan, France)
70,000 BAC clones (*c.a.* 110 kb) => 7.7 Gb
~ 2x coverage of hexaploid genome

Identification of homo(eo)logous BAC clones :

1) Target genomic regions:
- Region homologous to the *Sorghum Alcohol dehydrogenase* ***Adh1*** region, which was previously shown to be rearranged in grasses, but never explored in the Chloridoideae subfamily containing *Spartina*.
- Region containing the *cinnamyl alcohol dehydrogenase* ***(CAD)*** gene that is involved in the lignin biosynthetic pathway.

2) Probe design from orthologous sequence alignments:



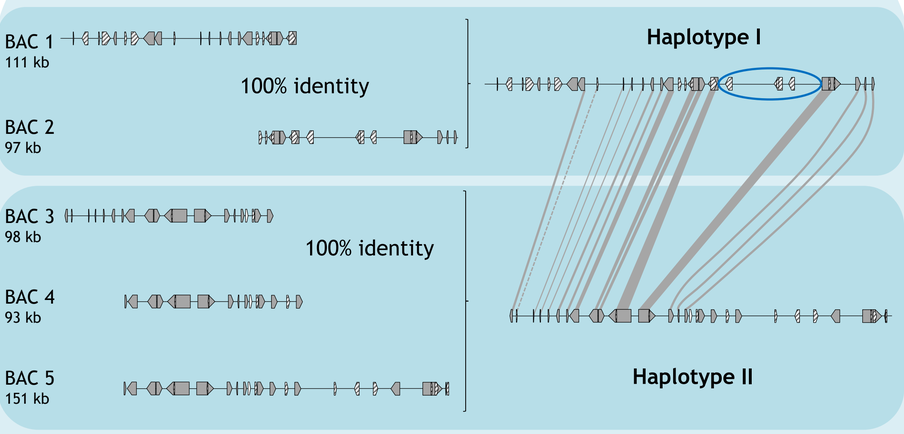
3) BAC clone sequencing and assembly (Génoscope)

4) Automatic and manual annotations of genes and transposable elements (GNPannot pipeline: http://southgreen.cirad.fr/ + Artemis annotation tool)

RESULTS

1. MICRO-SYNTENY ANALYSIS AND SEQUENCE HETEROGENEITY IN *S. MARITIMA* ADH1 AND CAD6 REGIONS

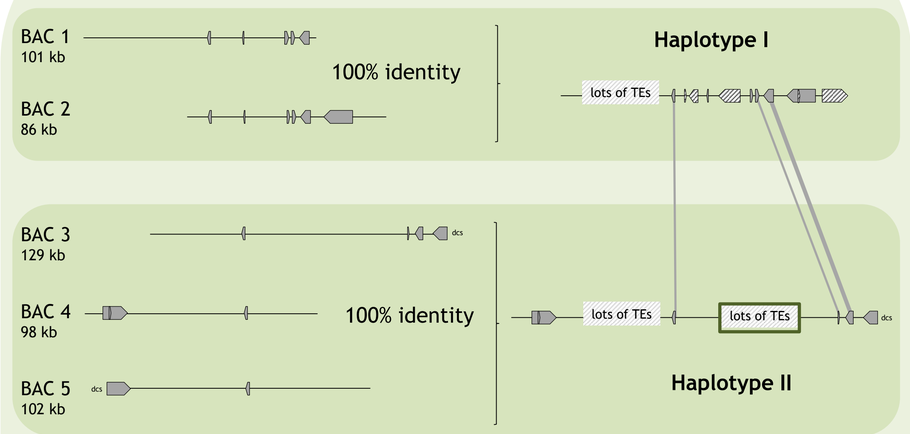
Adh1 region



Genes; Collinear homoeologous genes; Transposable elements (TEs); Collinear homoeologous TE

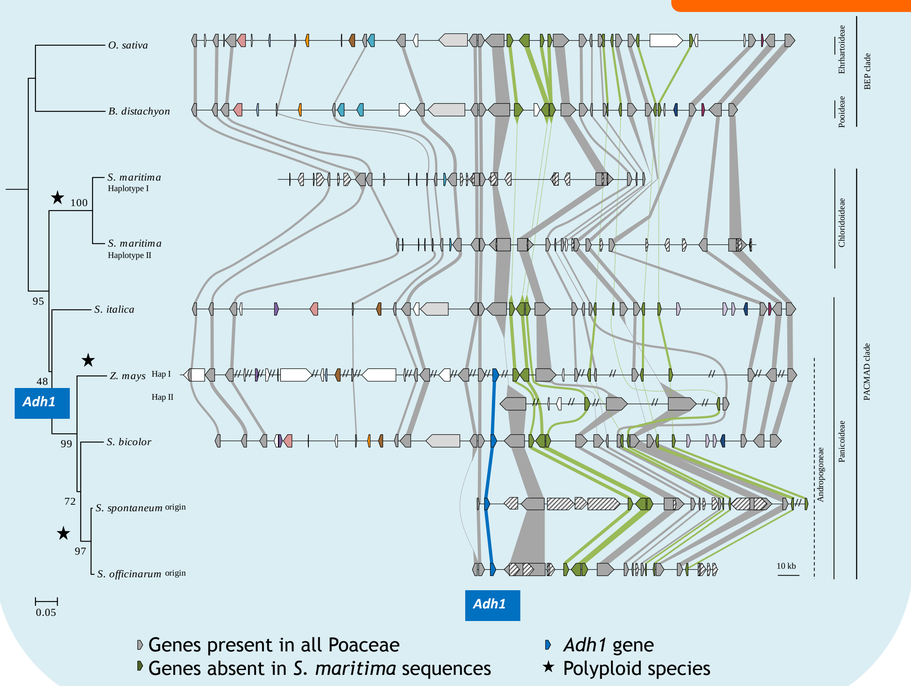
- For each of the analyzed regions, **two divergent homoeologous sequences were distinguished in *S. maritima*.** They exhibit **differential retro-element insertions** and an **average sequence identity of 96% in exons and 91% in introns.**
- **Perfect gene collinearity between homoeologs was observed for the Adh1 region whereas the CAD6 region appeared more rearranged.**

CAD6 region



Genes; Collinear homoeologous genes; Transposable elements (TEs)

2. COMPARISON WITH ORTHOLOGOUS REGIONS IN POACEAE



- **Local comparative analyses between *Spartina* and other grasses** allowed examining synteny relationships and detecting structural rearrangements that occurred following divergence of the different grass lineages.
- We show that **the translocation previously detected in the Panicoids (*Sorghum*, *Saccharum*, *Zea*) and containing the *Adh1* gene is not shared with *Spartina*, suggesting that this event took place after the divergence between Panicoideae and Chloridoideae (45-60 Mya).**
- **High level of gene collinearity among orthologs was observed for the Adh1 region whereas the CAD6 region appeared much more rearranged.**

CONCLUSION

These investigations of homoeologous genomic regions in *Spartina* provide the first estimates in terms of divergence and microsynteny in a hexaploid *Spartina* species. Two of three expected homoeologous regions were observed in *S. maritima*. Several hypotheses may explain these results, including incomplete BAC sampling or long term evolution of the duplicated genome (e.g. gene-loss-fractionation process). Comparative analyses at larger scale, including data from Next Generation Sequencing will allow exploring these hypotheses.

-Fortune PM, Schierenbeck KA, Ainouche AK, Jacquemin J, Wendel JF, Ainouche ML. 2007. Evolutionary dynamics of Waxy and the origin of hexaploid *Spartina* species (Poaceae). Mol Phylogenet Evol 43(3):1040-55.

- Ainouche ML, Chelaifa H, Ferreira de Carvalho J, Bellot S, Ainouche AK, Salmon A. 2012. Polyploid evolution in *Spartina:* dealing with highly redundant genomes. In: Soltis PS, Soltis DE (eds). Polyploidy and Genome Evolution. Springer: Berlin, Heidelberg, pp 225-244.